ASSOCIATION OF SERUM LEPTIN WITH ESSENTIAL HYPERTENSION IN ELDERLY MALES

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CERTIFICATE

This is to certify that the dissertation titled **"ASSOCIATION OF SERUM LEPTIN WITH ESSENTIAL HYPERTENSION IN ELDERLY MALES"** is the bonafide original work of **Dr.M.VIJAYALAKSHMI,** in partial fulfillment of the requirements for M.D., (Biochemistry), Branch - XIII, Examination of the Tamil Nadu Dr.M.G.R. Medical University to be held in March 2008.

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CONTENTS

INTRODUCTION

Aging is associated with a metabolic decline characterized by the development of changes in fat distribution, obesity, insulin resistance, decrease in elasticity, large artery thickening and stiffness as well as endothelial dysfunction[1,2]. All these metabolic alterations are associated with a variety of age related diseases that subsequently result in increased mortality[3].

It has been recently demonstrated that Leptin, a 16 kDa fat-derived peptide, can modulate many of these metabolic alterations characteristic of aging[4]. Leptin is an adipocyte-derived hormone that regulates food intake and energy expenditure[5]. It is believed to be of importance in regulation of body weight. Leptin acts on the brain, through specific receptor, to suppress synthesis and secretion of neuropeptide Y, the most potent stimulator of food intake and through various downstream mechanisms[6].

Serum leptin concentration is found to be associated with Body Mass Index (BMI) and more precisely with body fat mass. Mutations in the gene encoding Leptin was found to cause obesity in both animals and humans, indicating a relation between the leptin gene and obesity, though the frequency was less[7].

Leptin has also extra hypothalamic functions and potential sites of action that corresponds to the distribution of leptin receptor. In addition to its effect on food intake & energy expenditure, a direct effect of leptin on blood pressure has recently been reported[8,9]. High plasma leptin levels act as a pathophysiologic trigger of high blood pressure. A highly polymorphic

tetranucleotide repeat polymorphism in the 3' flanking region of the leptin gene has been identified. This polymorphism is associated with hypertension independent of obesity. Studies have also shown that chronic infusion of leptin in rats has resulted in elevated arterial pressure and heart rate[10,11].

Data from animal studies clearly indicate an association between leptin and hypertension, but results of human study are less consistent. Whether the association is independent of other factors such as insulin, obesity and renin-angiotensin systems is not known and needs to be investigated.

The main objective of the present study is to evaluate the association of leptin levels with essential hypertension in the non-obese elderly subjects.

REVIEW OF LITERATURE

Aging is a maturation process. It is thought to have the positive component of development and the negative component of decline. The changes that occur with aging can be categorized as those that result from aging itself and those that result from diseases, lifestyle and exposures[12].

Usual aging refers to the common complex of diseases and impairments that occur in many elderly people. However this complex is hard to define because people age very differently. Successful aging refers to a process by which deleterious effects are minimized, preserving function until senescence makes life impossible.

DISEASE Vs AGING

With aging many physiological functions decline. Many of these declines are attributed to aging itself, not disease-related. The distinction between normal and disease related may be clear or may simply be defined by statistical distribution. For example, some degree of glucose intolerance is considered part of normal aging, but diabetes, although very common, is considered a disease.

CARDIOVASCULAR PHYSIOLOGY IN AGING

With aging, there are changes in the cardiovascular system, which results in alterations in cardiovascular physiology. These changes must be differentiated from the effects of pathology, such as coronary artery disease that occur with increasing frequency as age advances. The changes with age

occur in everyone but not necessarily at the same rate, therefore accounting for the differences seen in people between chronological age and physiological age.

With aging, there is decrease in the elasticity and increase in the stiffness of the arterial system. This results in increased afterload on left ventricle. This results in increase in systolic blood pressure and left ventricular hypertrophy as well as other changes in the left ventricular wall that prolongs relaxation of the left ventricle in diastole. With fibrosis of the cardiac skeleton, there is calcification at the base of the aortic valve and damage to the His Bundle as it perforates the right fibrous trigone. Finally there is decreased responsiveness to β-adrenergic receptor stimulation, a decreased reactivity to baroreceptors and chemoreceptors and an increase in circulating catecholamines. These changes set the stage for all diseases seen in elderly.

Differences between cardiovascular function in older and younger persons have been quantified. However, interactions between age, disease and lifestyle are often overlooked. Whether the prevalence of cardiovascular disorders such as hypertension, coronary artery disease and heart failure is due to an aging process or these disorders merely occur more frequently in elderly persons because of a longer exposure to risk is not yet established.

CHANGES IN CARDIOVASCULAR STRUCTURE DURING AGING

The Heart

A modest increase in left ventricular wall thickness is normal with age; an exaggerated increase occurs in persons with hypertension. Other normal age

associated changes include enlargement of left atrium and slight enlargement of left ventricular cavity and of the cardiac silhouette.

The amount of fibrous tissue within the myocardium increases with age but does not contribute to cardiac mass, rather myocardial wall thickening occurs largely because cardiac myocytes increase in size. Some myocytes are replaced by fibrous tissue, so that the number of myocytes decreases with age.

THE VASCULATURE

With age, the walls of large distributing arteries thicken and the arteries become dilated and elongated. The thickening results mainly from an increase in intimal thickness due to cellular accumulation, matrix deposition and fragmentation of internal elastic membrane.

Collagen also increases and changes in the cross-linking of collagen in vascular media may make it less elastic. Glycoprotein eventually disappears from elastin fibrils and elastin becomes frayed. The total MPS content (ground substance) of interstitial matrix is unaltered with age but the amount of dermatan and heparan sulphate contained in the matrix increases and that of hyaluronate and chondroiton sulphate decreases.

CHANGES IN CARDIOVASCULAR FUNCTION DURING AGING

Compliance

With age, there is reduction in ventricular compliance.

Cardiac filling and Preload

The early diastolic left ventricular filling rate progressively slows after age. This reduction is attributed to structural changes in left ventricular myocardium or due to prolonged isovolumic relaxation. But the late diastolic filling is greater because the atrial contraction is augmented. The augmented contraction is accompanied by atrial enlargement and is manifested as fourth heart sound.

After load

After load varies from person to person and depends on peripheral vascular resistance, aortic impedance and aortic pulse wave velocity. With age, peripheral vascular resistance increases, aortic impedance increases and also the pulse wave velocity. As a result pressure waves from peripheral sites are returned to heart more quickly. Arterial stiffening and late augmentation of systolic blood pressure explains the overall increase in systolic blood pressure with age. Also it reflects the resetting of the baroreceptor reflex to a higher level.

Myocardial contractility

It involves ionised calcium activation of myofilaments (contraction-excitation coupling). Age associated changes are related to alterations in gene expression. The increase in myoplasmic calcium after excitation at low rates and affinity of myofibrils for calcium do not change with age. At higher rates of excitation, the amplitude of calcium transient is not well documented.

Relaxation is prolonged in senescent cardiac muscle, because calcium is removed more slowly from myoplasm during diastole. This slow removal probably occurs because the sarcoplasmic reticulum sequesters less calcium. A reduction in myocardial relaxation rate results in less complete myocardial relaxation when mitral valve opens and in a increase in early diastolic left ventricular filling rate.

Ejection fraction and Stroke volume

The resting ejection fraction is not reduced with age. Resting stroke volume increases slightly in older men but remains constant in older women.

Heart rate

The supine resting heart rate does not change, but the heart rate while seated decreases slightly in men and women. The intrinsic sinus rate decreases significantly with age.

Cardiac output

The resting cardiac index is not reduced in healthy age men. But in older women, resting cardiac output decreases slightly because neither end-diastolic volume nor stroke volume increases to compensate for the modest reduction in heart rate.

There is much evidence supporting the hypothesis that age associated changes in cardiovascular structure and function are related to the markedly increased risk for cardiovascular disease in older persons. Some vascular changes that occur with aging are risk factors for an increase in systolic blood

pressure and diastolic blood pressure and for the development of atherosclerosis and stroke.

Hypertension

Hypertension is very common as age advances. The morbidity and mortality of hypertension poses a great challenge globally for the society and for the health care professionals. It is the most important modifiable risk factor for coronary heart disease, stroke, congestive heart failure, end-stage renal disease and peripheral vascular disease.

With increasing age of general population, the prevalence of hypertension and its complications are likely to increase rather than decrease. The detection and treatment of hypertension is therefore, vital in the battle to reduce cardiovascular disease and stroke, which are currently the major cause of death[13].

Epidemiology

Hypertension is an important public health challenge because of its commonness and concomitant risks of cardiovascular disease. The estimated total number of adults with hypertension in 2000 was 972 million. Of these 333 million were estimated in economically developed countries and 639 million in economically developing countries. Europe – 37.4%, China – 22.6%, Australia – 37.4%, Africa – 26.9%, South America – 40.7% and North America – 37.4%[14].

Definition

The more recent guidelines from the American Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure (JNC VII)[15] define hypertension as a systolic blood pressure of 140mm Hg and/or a diastolic blood pressure of greater than 90mm Hg.

The definition of hypertension is necessarily arbitrary and varies between expert groups. However, it can be defined pragmatically as that level of blood pressure above which the use of anti-hypertensive treatment does more good than harm. This level will vary from patient to patient and balances the risks of untreated hypertension with those of long term exposure to anti-hypertensive drugs and their side effects[16].

Guidelines & Classifications

Hypertension is one of the conditions for which disease-specific guidelines have been generated by different organizations. The more recent guidelines from the American Joint National Committee on prevention, detection, evaluation and treatment of high blood pressure (JNC VII) is given in Table1.

Aetiology of hypertension

There are two types:

a. Primary or essential hypertension – over 95% of patients with hypertension in whom no immediately obvious underlying

causewas present. It is a product of genetic predisposition with environmental and lifestyle factors.

Genetic predisposition – A family history of hypertension, heart disease, type2 diabetes mellitus

Environmental factors – Age, hormone state

Lifestyle factors – Smoking, heavy drinking, overweight, sodium and calorie rich diet, lack of physical activity, stress

b. Secondary hypertension – 2 to 5%; has an underlying cause, usually involving the kidneys and the endocrine system

CAUSES

1. Endocrine

Adrenocortical hyperfunction –

* Cushings' disease and syndrome
* Primary hyperaldosteronism
* Congenital adrenogenital syndrome (17α-hydroxylase and 11β hydroxylase defects)
* Myxedema
* Acromegaly

2. Pheochromocytoma

3. Vascular – Coarctation of aorta

Renal artery stenosis or renal infarction

4. Renal

   Intrinsic renal disease (glomerulonephritis)

   Polycystic kidney

   Chronic renal failure

5. Drugs Oral contraceptives

   Sympathomimetics

COMPLICATIONS OF HYPERTENSION

a. Stroke – It is the most devastating consequences of hypertension. In patients with hypertension, about 80% of strokes are ischaemic, caused by intra-arterial thrombosis or embolisation from the heart and large arteries. The remaining 20% are from haemorrhagic causes, which may also be related to high blood pressure[15].

b. Coronary artery disease – Fatal coronary artery disease is seven times more common among hypertensives than fatal stroke. Controlled trials have consistently shown that stroke and heart failure are largely preventable by treatment of high blood pressure but the reduction of coronary thrombosis is less impressive. Adequate treatment reduces heart attack risk by approximately 20%.

c. Left ventricular hypertrophy – As a result of increased after load imposed on the heart by high blood pressure, the mass of left ventricular muscle increases. While this is initially a

compensatory response, the increased muscle mass outstrips its oxygen supply and coupled with the reduced coronary vascular reserve seen in hypertension, can result in myocardial ischemia even with normal coronary arteries. Thus, left ventricular hypertrophy secondary to hypertension is a major risk factor for myocardial infarction, stroke, sudden death and congestive cardiac failure.

d. Large vessel arterial disease – Peripheral vascular disease as manifested by intermittent claudication is three times more common in patients with hypertension. Atheromatous disease in the aorta, coupled with hypertension, may progress to aortic aneurysm. Extracranial carotid artery disease is also more common and is one of the mechanisms by which hypertension leads to the increased risk of stroke.

e. Renal disease – Malignant hypertension frequently leads to progressive renal failure. There is some controversy as to whether this is also the case in patients with mild to moderate essential hypertension.

f. Retinopathy – Hypertension leads to vascular changes in the eye, referred to as hypertensive retinopathy. These changes have been classified by Keith, Wagener and Barker into four grades. The most severe form – malignant hypertension – is defined clinically as raised blood pressure in association with bilateral retinal flame-shaped haemorrhages and/or cotton wool spots and/or hard exudates with/without papilledema.

PATHOGENESIS

The pathogenesis of essential hypertension is multifactorial and highly complex. Multiple factors modulate the blood pressure for adequate tissue perfusion. They include humoral mediators, vascular reactivity, circulating blood volume, vascular caliber, blood viscosity, cardiac output, blood vessel elasticity and neural stimulation[17]. Multiple factors have been proposed as possible pathogenesis of essential hypertension. They include

HEREDITY

Genetic factors have long been assumed to be involved in the genesis of hypertension. Most studies support the concept that the genetic inheritance is probably multifactorial or that a number of different genetic defects each have an elevated blood pressure as one of their phenotypic expressions. Both monogenic defects and susceptibility genes have now been reported which has one of their consequences an increased arterial pressure.

ENVIRONMENT

A number of environmental factors including salt intake, obesity, alcohol intake, family size all have been assumed to be important in the increase in blood pressure with age.

SALT SENSITIVITY

This environmental factor has received the greatest attention. It illustrates the heterogeneous nature of the essential hypertensive population, in

that the blood pressure is only approximately 60% of hypertensives are particularly responsive to the level of sodium intake.

ROLE OF RENIN

Renin is an enzyme secreted by the juxtaglomerular cell of the kidney and linked with aldosterone in a negative feedback loop. Its rate of secretion depends on the volume status of the individual. The end product of the action of renin on its substrate is the generation of angiotensin II. The response of target tissues to this peptide is uniquely determined by the prior dietary electrolyte intake.

CELL MEMBRANE DEFECT

Generalised cell membrane defect is one possible explanation. This hypothesis derives most of its data from studies on circulating blood elements, in which abnormalities in the transport of sodium across the cell membrane have been documented. This defect occurs in many cells of the body particularly the vascular smooth muscle cells, which leads to an abnormal accumulation of calcium in the vascular smooth muscle leading to increased vascular reactivity.

INSULIN RESISTANCE

Insulin resistance and/or hyper insulinaemia have been suggested to be responsible for the increased arterial pressure. Four mechanisms have been postulated.

a. Insulin has mitogenic action, which increases vascular smooth muscle hypertrophy.

b. It produces renal sodium retention and increases sympathetic activity, which increases arterial pressure.

c. Insulin also modifies ion transport across the cell membrane, increasing the cytosolic calcium levels of insulin-sensitive vascular or renal tissues.

Recently studies have demonstrated that high plasma leptin levels are associated with high blood pressure in the elderly[18]. Data from available animal studies clearly indicate an association between leptin and hypertension, whereas results of human studies are less consistent. There are several possible mechanisms, which include

a. One possible explanation is through sympathetic activation. Leptin administered intravenously and intracerebroventricularly in rodents has been found to increase the sympathetic outflow to kidneys, skeletal muscle vasculature and the neural traffic to the adrenal[19,20].

b. It induces endothelin-1, which is a potent vasoconstrictor and mitogen[21]

c. It promotes angiogenesis, which contributes to the modulation of endothelial cell proliferation in atherosclerosis[22].

d. It increases sodium and water excretion via a direct tubular action. But during leptin resistance it produces anti-natriuresis leading to hypertension[23].

Diagnosis

An accurate measurement of the blood pressure is the key to diagnosis. At any given visit, an average of three blood pressure readings taken two minutes apart using a mercury manometer is preferable. Blood pressure should be measured in both the supine and sitting positions, auscultating with the bell of the stethoscope. Improper cuff size may influence blood pressure measurement and hence wider cuff is preferable. The patient should rest quietly for at least five minutes before the measurement. Palpation of all the peripheral pulses should be performed.

Laboratory studies

Unless a secondary cause for hypertension is suspected, only the following routine laboratory studies should be performed.

a. Complete Blood Count, serum electrolytes, serum creatinine, plasma glucose, uric acid and urine analysis.

b. Lipid profile

c. Electrocardiogram

Treatment

Lifestyle modifications – JNC VII recommendations to lower blood pressure and decrease cardiovascular disease risk include the following[24-27].

- Weight reduction
- Limit alcohol intake and smoking
- Increase physical activity
- Reduce sodium intake to no more than 6 gms
- Adequate intake of dietary potassium, calcium and magnesium
- Reduce intake of dietary saturated fats and cholesterol

Initial therapy based on the JNC VII report recommendations is as follows

* Pre-hypertension (systolic –> 120-139, diastolic –> 80-89): No anti-hypertensive drug is indicated

* Stage I hypertension (systolic –> 140-159, diastolic –> 90-99): Thiazide-type diuretics are recommended. ACE inhibitor, beta blocker, calcium channel blocker or combination may be considered

* Stage II hypertension (systolic > 160, diastolic > 100): Two drug combination (Thiazide - type diuretic and ACE inhibitor or beta blocker is recommended).

Prevention

A comprehensive strategy for reduction in mortality and morbidity from hypertension must include prevention strategies, earlier detection and adequate treatment.

Even a small reduction in blood pressure confers significant health benefits. A 2mm Hg reduction in diastolic pressure is estimated to decrease the risk of stroke by 15% and the risk of coronary artery disease by 6%. Prevention may be achieved by

- Weight control
- Increased physical activity
- Moderate sodium and alcohol intake
- Increased potassium intake
- A dietary pattern rich in fruits and vegetables, low fat meat, fish and dairy products

AN OVERVIEW OF LEPTIN STRUCTURE, GENE AND EXPRESSION

Leptin (Greek leptos, "then") is a small protein that is produced in adipocytes. It is a product of gene designated OB (Obese). The gene has 3 exons separated by 2 introns in both rodents and humans. The coding region of the gene is contained in exon 2 & 3 separated by an intron, approximately 2 kb. Considine et al[28] reported the mRNA of the OB gene is highly expressed in adipocytes. This OB mRNA expression is not detected in human pre-adipocytes but occur following differentiation of 373L1 and 373F, 422A cells

from fibroblasts to mature adipocytes as found by Leroy & co-workers[29,30]. The gene is located in Chromosome 6 in mice and Chr.7q.31.3 in humans[31].

Protein product of OB gene is a 167 amino acid protein with an amino terminal secretory signal sequence of 21 amino acids. The signal sequence is removed subsequently in the secretory pathway[32]. Leptin circulates as 146 amino acid protein. Its secretion is constitutive. There is no storage form[33]. It is a globular protein with a tertiary structure similar to hemopoietic cytokines such as interleukin and granulocyte macrophage colony stimulating factor[34]. The size of the adipocyte appears to be the major determinant of ob mRNA, as larger cells contain more ob mRNA than smaller cells isolated from the same subject[35].

Leptin Receptors

Leptin Receptor OB-R belongs to Class I cytokine receptor family and atleast six various alternatively spliced forms of leptin receptor mRNA have been reported[36]. The short length OB-Ra receptor is present in all tissues but its functional significant activity is not known.

a. In the central nervous system: OB-Ra receptor is expressed in the choroid plexus and this may transport leptin across the blood brain barrier to the cerebrospinal fluid[37]. In choroid plexus, leptin receptor consists of 894 amino acids with 22 amino acid secretory signal sequence, 23 amino acid transmembrane domain and a short cytoplasmic domain of 34 amino acid.

A second leptin OB-Rb receptor is found in hypothalamus and is identical to that in choroids plexus except that the intracellular domain has additional 269 amino acids. The extra cellular domain contains Trp-Ser-X-Ser-Trp motifs and the intracellular domain contains the Janus Kinase interaction motif and a signal transducer and activator of transcription (STAT) motif. OB-Rc and OB-Rd are speculated that they transport leptin across the blood brain barrier. The soluble OB-Ra receptor functions as a transport protein contributing to binding and activation of circulating leptin[38,39].

b. In peripheral organs: The mRNA of several leptin isoforms has been found in non-neuronal tissues such as the pancreas, kidney, liver and in the reproductive and haematopoietic organs[40]. Leptin is involved in pathways other than energy metabolism as a true pleotropic hormone mediating a variety of peripheral action[41,42].

Leptin binding

Leptin has been found to bind competitively to atleast three serum macromolecules with molecular masses of 85, 176, 240kDa in rodents and to 176 and 240 kDa in humans[43]. Leptin binding proteins are possibly soluble forms of leptin receptors. In non-obese humans, significant endogenous leptin is bound when the leptin level increases as in case of obesity free unbound leptin spills over into bioactive free protein pool. Recently it has been demonstrated that oxidized form of α2 macroglobulin binds competitively with leptin[44,45].

Leptin receptor and signal transduction

Leptin is structurally related to cytokines and acts on receptors that belong to the cytokine receptor superfamily. Several different leptin receptor isoforms exists including a long form (OB-Ri), which is highly expressed in hypothalamus[46,47].

It activates cytokine-like signal transduction via OB-Ri[48]. Upon leptin stimulation, intracellular Janus tyrosine kinases (JAKs) are activated via transphosphorylation and phosphorylates tyrosine residues on the long form of leptin receptor and on signal transducers and activators of transcription (STAT) proteins[49]. Phosphorylated STAT proteins dimerize and translocates to the nucleus to activate gene transcription. Lack of functional leptin in lep^{ob}/lep^{ob} mice or lack of the intracellular domain of OB-Ri in db/db mice produces severe obesity[50]. In addition, leptin receptor activation stimulates both the phosphotidyl-inositol-3-kinase (PI3K) and Ras-mitogen activated protein kinase (MAPK) signaling pathways, both of which are downstream of JAK[51].

The arcuate nucleus (ARC) of the hypothalamus serves as the leptin signaling center. Leptin targets two adjacent pathways within ARC, the appetite- stimulating (Orexigenic) pathway mediated by neuropeptide Y and agouti-related protein (AgRP) and the appetite-suppressing (anorexigenic) pathway mediated by pro-opiomelanocortin via the Ob-Rb form of leptin receptor[52]. When leptin binds to its receptor, a signaling cascade is initiated, activating PI3K. This causes a conversion of F-actin to G-actin, which subsequently opens K^+ATP channels.

Leptin resistance

Potential mechanism for leptin resistance include defects in transport of leptin across the blood brain barrier, defects in leptin signal transduction in leptin-receptor expressing neurons in the hypothalamus and antagonism of leptins physiologic actions at one or more steps beyond initial leptin-responsive neurons.

Recently, a new family of cytokine inducible inhibitors of signaling has been identified including CIS (Cytokine-inducible sequence), SOCS-1 (Suppressor of cytokine signaling), SOCS-2 and SOCS-3. CIS and SOCS are small proteins having a central SH2 domain and a 40 aminoacid long carboxy terminal SOCS box[53]. The SH2 domain of SOCS is thought to bind to phosphorylated tyrosine residues on JAK proteins, while SOCS box plays a role in degradation of SOCS proteins[54].

Leptin specifically induces expression of SOCS-3 mRNA in regions of hypothalamus that expresses OB-Ri[55]. Forced expression of SOCS-3 blocks leptin- receptor mediated signal transduction by attenuating leptin- induced JAK-2 tyrosine phosphorylation. Thus SOCS-3 plays a major role in negative regulation of proximal leptin signal transduction.

Physiological functions of leptin

a. Effect on body fat mass

Body weight is a simple non- invasive predictor of body fat and caloric balance. Quetelet Index / Body Mass Index is a good index of body fat in both men and women.

$$BMI = \frac{W \text{ (Weight in Kg)}}{H^2 \text{ (Height in m)}}$$

Values greater than 30 represent obesity. Other methods for measuring body fat mass include Waist / Hip ratio, bio-electric impedance analysis, subcutaneous tissue biopsy and skin fold thickness. Serum leptin is highly correlated with percent body fat and with BMI in humans[56].

Leptin is secreted by adipocytes and circulates in the blood in concentrations proportional to fat mass content. Interaction of leptin with its receptor in the hypothalamus inhibits food intake and increases energy expenditure, that leads to a reduction in adipose tissue mass[57].

The mean serum leptin level in normal obese human is approximately four fold higher than that detected in healthy lean subjects. Some earlier studies have found that leptin level is independent of body fat[58]. For a given BMI, women have leptin concentration approximately twice that of men that remains even after correction for percentage body fat. Testosterone is responsible for this gender difference[59].

b. Effect on carbohydrate and lipid metabolism

Leptin directly inhibits intracellular lipid concentrations by reducing fatty acid and triglyceride synthesis and concomitantly increasing lipid oxidation[60]. This is mediated by an inhibitory effect of leptin on acetyl-CoA carboxylase activity, the rate-limiting enzyme in fatty acid synthesis[61].

Inhibition of this enzyme leads to a reduction in malonyl-CoA, an inhibitor of carnitine acyl transferaseI and mitochondrial β-oxidation.

Inhibition of acetyl CoA carboxylase will block fatty acid synthesis and favours mitochondrial uptake and oxidation, resulting in lower intracellular fatty acid and triglyceride concentration. Unger and coworkers suggest that leptin, by reversing lipid accumulation in several tissues, could have beneficial effects on insulin resistance and β-cell function, ultimately improving glucose homeostasis.

c. Effect on energy balance

Leptin increases energy expenditure by which it regulates body weight[62]. This involves increased thermogenesis in brown adipose tissue as well as increased sympathetic nerve activity and norepinephrine turnover. The key element is a mitrochondrial transport protein called Uncoupling Protein-1 (UCP-1) which causes uncoupling of respiration from oxidative phosphorylation without ATP synthesis. Leptin by increasing gene expression of UCP-1 in brown adipose tissue increases energy expenditure[63].

d. Action on central nervous system

It acts as a modulator of appetite via a number of hypothalamic mediators. Leptin decreases the level of mRNA for Neuropeptide Y (NPY) in the arcuate nucleus. It also increases the level of mRNA for an inhibitor of food intake corticotrophin releasing hormone in the paraventricular nucleus[64].

An intracerebroventricular injection of leptin increases the activity of both lumbar and renal sympathetic nerve activity and reduces arterial blood flow to skeletal muscle. Leptin also penetrates the blood-cerebrospinal fluid barrier by active transport and activates sympathetic nerve activity in the central nervous system. Leptin binding sites have been found in regions of the brain that are also important in cardiovascular control, hence there is reason to believe that leptin could affect cardiovascular function through its effect on the central nervous system.

e. Regulation of insulin and glucose homeostasis

Leptin markedly decreases plasma insulin concentration and modestly reduces blood glucose. The mechanisms are not clear yet. A recent study by Kieffer et al indicates that there are leptin receptors located on the pancreatic β cells, although the function of these receptors are not known[65]. Leptin may also decrease insulin release by stimulating α-adrenergic receptors in the pancreas via its effect on sympathetic activity. But the finding that it reduced plasma glucose suggests additional effects besides a simple inhibition of insulin secretion.

Another possibility is that leptin increases glucose utilization or improved insulin sensitivity in peripheral tissues, which enhances glucose disposal in skeletal muscle and fat cell and suppresses glucose output by the liver. Thus the decrease in plasma insulin could be a compensatory response to a fall in plasma glucose.

f. Renal effects

The kidneys express full length leptin receptor. It can be speculated that insulin and leptin interact and modulate each other's effect on renal sodium handling. Insulin infusion causes anti-natriuresis in healthy subjects, whereas leptin infusion increases renal, sodium and water excretion via a direct tubular action without affecting renal blood flow and glomerular filtration rate[66]. It can be speculated that some of the peripheral actions of leptin such as diuresis and natriuresis act as compensatory mechanisms against the potential deleterious effects of an increased fat mass.

Kidneys play an important role in leptin metabolism because leptin is removed from the circulation primarily by the kidneys. In renal failure, there is reduced renal clearance, which contributes to elevated plasma leptin concentration.

Recent in-vitro studies suggest that leptin could induce proliferation and differentiation of haematopoietic stem cells and there might be synergism between leptin and erythropoietin[67,68].

g. Effect on cardiovascular system

Leptin levels cause sustained changes in cardiovascular and neurohumoral function. High leptin levels are found to elevate arterial pressure and heart rate. Studies have shown that chronic infusion of leptin in rats has resulted in elevated arterial pressure and heart rate. A highly polymorphic tetranucleotide repeat polymorphism in the 3' flanking region of the leptin gene

has been identified[11]. This polymorphism is associated with hypertension independent of obesity.

There are several mechanisms by which leptin increases the arterial pressure

1. One possible explanation is through sympathetic activation. Leptin administered intravenously, intracerebroventricularly and into hypothalamic nuclei in rodents has been found to increase the sympathetic outflow to the kidneys, adipose tissue and the skeletal muscle vasculature and the neural traffic to the adrenals[8].

2. It has also been reported that leptin induces endothelin-1, a potent vasoconstrictor and mitogen which causes vasoconstriction of glomerular afferent and efferent arterioles which leads to decreased renal plasma flow and GFR[21].

3. It also contributes to hypertension via its effects on tubular sodium handling[23].

4. It also promotes angiogenesis, which contributes to the modulation of endothelial cell proliferation in atherosclerosis[22].

AIM OF THE STUDY

On reviewing the physiological role of leptin and its association with essential hypertension in the elderly patients, the study has been taken up with the keen interest to establish the following aims:

* To determine the reference ranges for the study for the following parameters: fasting plasma glucose, serum urea and creatinine, lipid profile including serum triglycerides (TGL), total cholesterol (TC), HDL-C and LDL-C.

* To determine the reference range of serum leptin levels for the study.

* To determine the level of serum leptin levels in subjects with essential hypertension.

* To analyze whether the leptin levels in essential hypertension varied from the above range.

* To analyze the level of lipid parameters in patients with essential hypertension and to determine whether there is any significant change of its level from that of apparently healthy controls.

* To analyze the body mass index and waist hip ratio in patients with essential hypertension and to determine whether there is any significant change in the ratio from that of apparently healthy controls.

MATERIALS AND METHODS

The study was carried out during the period Jan 2007-April 2007. It was done in 2 groups, namely, apparently healthy controls and subjects with essential hypertension in the elderly males (> 60 years).

Control group

The group comprised of 39 apparently healthy elderly male subjects with no significant medical illness. They were selected from the patients attending the outpatient department (OPD) of Geriatrics, Madras Medical College, Chennai

Test group

This group comprised of elderly males with essential hypertension and were attending the OPD of Geriatrics, Madras Medical College, Chennai. Based on the following inclusion and exclusion criteria males of this group were selected.

Inclusion criteria

Patients with confirmed diagnosis of essential hypertension based on history and blood pressure measurement using sphygmomanometer.

Exclusion criteria

* Patients of essential hypertension with associated diabetes mellitus

* Patients of essential hypertension without any h/o coronary artery disease and any other medical illness.

* Patients of essential hypertension with high body mass index.

Sample collection

6 ml of peripheral venous blood was withdrawn from all the study subjects under sterile conditions with disposable syringes after overnight fasting. One ml of blood was transferred into the test tube containing a pinch of potassium oxalate and sodium fluoride (3:1 mixture) for plasma glucose estimation. The remaining 5 ml of blood was transferred to a plain tube.

Serum separated from this tube was pipetted into a centrifuge tube and was centrifuged at 2500 revolutions per minute for 5 minutes, to get clear serum without any cells.1 ml of the above serum was stored at –20°C for the estimation of serum leptin. From the remaining serum, parameters such as Urea, Creatinine, serum triglycerides, total cholesterol, HDL and liver function tests were measured within 6 hours of blood collection by enzymatic methods using commercial kits. Height (in cms) and Weight (in kgs) of the subjects were measured to calculate the body mass index. Waist and hip circumference were measured to calculate waist/ hip ratio.

The biochemical parameters undertaken for the study were determined using the following methodologies:

ESTIMATION OF SERUM LEPTIN LEVELS – ELISA METHOD

Samples

In the present study serum samples were stored at -20^{o}c and analysed within 3 months.

Available methods

Earlier assays were semi quantitative based on immunoprecipitation. Western blot technique uses an immunopurified biotinylated primary antibody raised by immunizing rabbit with a peptide corresponding to the first 20 amino acids of leptin. The first commercial assay was produced in 1996 by Linco Research Inc., Missouri, OSA as detailed by Ma et al.

The Linco assay utilizes I^{125} labeled human recombinant leptin, human recombinant calibrators and an antiserum raised by immunizing rabbits with highly purified recombinant human leptin. By this kit, leptin level can be measured in serum, plasma, cerebrospinal fluid, tissue culture media.

Enzyme Linked Immuno Sorbent Assay (ELISA) is also available.

HUMAN LEPTIN ASSAY BY ELISA

Principle

It is a solid phase Enzyme Linked Immuno Sorbent Assay (ELISA) based on the sandwich principle.

The microtiter wells are coated with a monoclonal antibody directed towards a unique antigenic site on a leptin molecule. An aliquot of patient sample containing endogenous leptin is incubated in the coated well with a specific rabbit anti-leptin antibody. A sandwich complex is formed. After incubation the unbound material is washed off and an anti rabbit peroxidase conjugate is added for detection of the bound leptin.

Having added the substrate solution, the intensity of colour developed is proportional to the concentration of leptin in the patient sample

Reagents

Human leptin ELISA kit was procured from DRG Instruments GmbH, Germany. Each kit has 96 microtiter wells and contains the following reagents.

1. Microtiter wells 12*8 (break apart) strips, 96 wells; Wells coated with antileptin antibody (monoclonal)

2. Standard (0-5), 6 vials, 200 µl, ready to use; contains 0.3% Proclin as a preservative.

3. Control, 2 vials, 200 µl, ready to use; 2 levels (low and high) contain 0.3 % Proclin as a preservative.

4. Assay buffer, 1 vial, 11 ml, ready to use; contains 0.3% Proclin as a preservative

5. Antiserum, 1 vial, 11 ml, ready to use; Polyclonal leptin antiserum, contains 0.3% Proclin as a preservative.

6. Enzyme complex, 1 vial, 11 ml, ready to use; Anti rabbit complex conjugated to horseradish peroxidase, contains < 0.3% proclin as a preservative.

7. Substrate solution, 1 vial, 11 ml, ready to use

8. Stop solution, 1 vial, 6ml, ready to use, contains 0.5 M H_2SO_4

9. Wash solution, Qty: 30ml/vial (40 x concentrated)

   Preparation: 30 ml of concentrated wash solution is diluted with 1170ml deionized water to a final volume of 1200ml.

Storage and Stability

All reagents and kits were refrigerated at 2-8 ^{o}c.

Assay procedure

Step 1: The desired number of microtitre wells was secured in the holder.

Step 2 : Using new disposable tips, 15µl of each standard, controls and samples were dispensed into appropriate wells.

Step 3 : 100µl Assay buffer was dispensed into each well.

Step 4 : Complete mixing was done for 10 seconds.

Step 5 : The plate was then incubated at room temperature for 120 minutes.

Step 6 : The contents of the wells were briskly shaked out followed by rinsing of wells 3 times with diluted wash solution (300µl per well). The wells were striked well on adsorbent paper to remove residual droplets.

Step 7 : Then 100µl of antiserum was added to each well and allowed to incubate for 30 minutes at room temperature.

Step 8 : The contents of the wells were briskly shaked out followed by rinsing of wells 3 times with diluted wash solution (300µl per well). The wells were striked well on adsorbent paper to remove residual droplets.

Step 9 : Then 100µl enzyme complex was added into each well and allowed to incubate for 30 minutes at room temperature.

Step 10 : The wells were then rinsed 3 times with wash solution (300µl per well) and residual droplets were removed with adsorbent paper.

Step 11 : Then 100µl of substrate solution was added to each well and allowed to incubate for 15 minutes at room temperature.

Step 12 : The enzymatic reaction was then stopped by adding 50µl of stop solution to each well.

Step 13 : The OD was read at 450±nm with a microtitre plate reader within 10 minutes after adding stop solution.

Calculation of Results

1. The average absorbance values for each set of standards, controls and patient samples were calculated.

2. A standard curve was constructed by plotting the mean absorbents obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.

3. Using the mean absorbance value for each sample, the corresponding concentration was determined from the standard curve.

Normal value

Males – 3.84 ± 1.79

Females – 7.36 ± 3.73

Assay characteristics

Assay Dynamic Range

The range of the assay is between 0-100ng/ml

Cross Reactivity

The assay had no detectable cross reactivity with Human Insulin, Human Proinsulin, Human C-Peptide, Glucagon and IGF-1.

Analytical Sensitivity

The analytical sensitivity was calculated from the mean plus two standard deviations of twenty replicate analysis of Standard 0 and was found to be 1.0ng/ml.

Estimation of Plasma Glucose

Method **:** Glucose oxidase peroxidase (GOD/POD)

Kit Used **:** Autopak of Bayer Diagnostics

Principle

$$\text{Glucose} + O_2 \xrightarrow{\text{Glucose Oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenolic compound} + \text{4 aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Pink coloured compound} + 2H_2O \text{ (Quinonemine)}$$

The intensity of pink coloured compound is proportional to glucose concentration and was measured at 505nm.

Reagents

1. Glucose reagent – Consists of glucose oxidase, peroxidase, 4aminoantipyrine, 4hydroxy benzoic acid and phosphate buffer

2. Glucose standard - 100mg/dl

Reagent reconstitution

Working solution was prepared by dissolving one tablet of glucose reagent in 20ml of deionised water with continuous stirring. It was stored in brown bottle.

Procedure

To 1ml of working solution, 10µl of plasma was added and incubated at 37^{o}c for 15 mins and absorbance was measured at 505.

Reference range

Fasting plasma glucose –> 70 –100 mg/dl

Estimation of BUN

Method : UV method

Kit used : Autopak of Bayer Diagnostics

Principle

Urea is hydrolysed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia produced combines with α-ketoglutarate and NADH in the presence of glutamate dehydrogenase to yield glutamate and NAD. The amount of urea nitrogen may be calculated by determining the absorbance decrease per minute relative to urea nitrogen standard at 340nm.

Reagents

Reagent 1(Enzymes)

ADP	0.66M mol/L
GLDH	$\geq$ 1000U/L

Urease	≥ 30000U/L
NADH	0.32mmol/L
α-Ketoglutarate	9 mmol/L

Reagent 1A(Buffer)

Tris buffer, pH 7.55	75mmol/L

Standard(BUN 20 mg/dl)

Urea	0.428g/L

Reagent reconstitution

Reagents were allowed to attain room temperature. One bottle of reagent 1 was mixed with one bottle of reagent 1A and mixed by gentle swirling.

Procedure

To 1 ml of the reconstituted reagent 10µl of serum is added and read immediately at 340 nm.

Reference Range

Serum / plasma : 5-25 mg/dl

Estimation of Serum Creatinine

Method **:** Picrate method

Kit used **:** Autopak of Bayer Diagnostics

Principle

Creatinine in alkaline solution reacts with picrate to form a red- orange compound. The colour is proportional to the concentration of creatinine in the sample when measured at 500nm.

Reagents

Reagent 1 (Picrate)

Picric acid	34.9mmol/L
Sodium hydroxide	45mmol/L

Reagent 2 (Sodium hydroxide)

Sodium hydroxide	0.26mol/L

Standard (Creatinine 2 mg/dl)

Creatinine	0.020 g/L

Reagent Reconstitution

Allow the reagents to attain room temperature. Mix equal volumes of reagent 1 and reagent 2 in a clean beaker.

Procedure

To 1ml of the reconstituted reagent 100µl of the serum is added and read immediately.

Reference Values

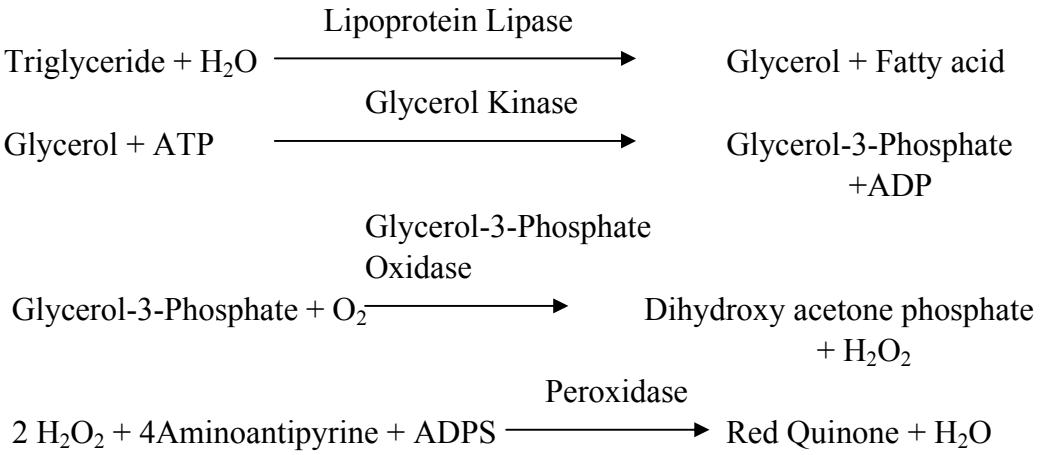
Males	: 0.6-1.1mg/dl
Females	: 0.5-0.9 mg/dl

Estimation of Serum triglyceride

Method **:** Enzymatic colorimetric method.

Kit used **:** Autopak of Bayer Diagnostics

Principle



The intensity of purple coloured complex formed during the reaction is directly proportional to the triglyceride concentration in the sample and is measured at 546nm.

Reagents

Reagent 1 (Enzymes / Chromogen)

Lipoprotein lipase	$\geq$ 1100 U/L
Glycerol kinase	$\geq$ 800 U/L
Glycerol-3-Phosphate Oxidase	$\geq$ 5000 U/L
Peroxidase	$\geq$ 350 U/L
4-Aminoantipyrine	0.7 mmol/L
ATP	0.3 mmol/L

Reagent 1A (Buffer)

Pipes buffer, pH 7.50	50 mmol/L
ADPS	1 mmol/L
Magnesium salt	15 mmol/L

Standard (Triglycerides 200 mg/dl)

Glycerol (Trig. equivalent) 2 g/L

Reagent Reconstitution

Reagents were brought to room temperature. Contents of one bottle of reagent 1 were dissolved with one bottle of reagent 1A. It was mixed gently by swirling.

Procedure

To 1 ml of the reconstituted reagent 10µl of serum is added and read at 546 nm after incubation at 37^{o}c for 5 min.

Reference range

Males : 60-165 mg/dl

Females : 40-140 mg/dl

Estimation of Serum Total Cholesterol

Method : Cholesterol Esterase – Cholesterol Oxidase

Kit used : Autospan of Span Diagnostics Ltd

Principle

$$\text{Cholesterol Ester} + H_2O \xrightarrow{\text{Cholesterol Esterase}} \text{Cholesterol} + \text{Fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{Cholesterol Oxidase}} \text{Cholestenone} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-Aminoantipyrine} \xrightarrow{\text{Peroxidase}} \text{Red quinone} + 4H_2O$$

The concentration of cholesterol in the sample is directly proportional to the intensity of the red complex (Red Quinone), which is measured at 500 nm.

Reagents

Reagent 1 (Enzymes / Chromogen)

Cholesterol Esterase	$\geq$ 200 U/L
Cholesterol Oxidase	$\geq$ 250 U/L
Peroxidase	$\geq$ 1000 U/L
4-Aminoantipyrine	0.5 mmol/L

Reagent 1A (Buffer)

Pipes buffer, pH 6.90	50 mmol/L
Phenol	25 mmol/L
Sodium Cholate	0.5 mmol/L

Standard (Cholesterol 200 mg/dl)

Cholesterol	2 g/L

Procedure

To 1ml of the reconstituted reagent, 10µl of serum was added and reading was taken after 5 mins of incubation at 37 ^{o}c.

Reference values

Cholesterol : 150-260 mg/dl

Estimation of HDL Cholesterol

Method **:** Phosphotungstate method

Kit Used **:** Autopak of Bayer Diagnostics

Principle:

Chylomicrons, VLDL (Very Low Density Lipoprotein) and LDL fractions in serum or plasma are separated from HDL by precipitating with phosphotungstic acid and magnesium chloride. After centrifugation, the cholesterol in the HDL fraction, which remains in the supernatant is assayed with enzymatic cholesterol method, using cholesterol esterase, cholesterol oxidase, peroxidase and the chromogen 4-aminoantipyrine/ Phenol.

Reagents

Reagent 1(Enzymes/ Chromogens)

Cholesterol esterase	$\geq$ 200U/L
Cholesterol oxidase	$\geq$ 250U/L
Peroxidase	$\geq$ 1000U/L
4-Aminoantipyrine	0.5 mmol/L

Reagent 1A (Buffer)

Pipes buffer, pH 6.90	50 mmol/L
Phenol	24 mmol/L
Sodium Cholate	0.5 mmol/L

Reagent 2(Precipitating Reagent):

Phosphotungstic acid	2.4 mmol/L
Magnesium Chloride	39 mmol/L

Standard (HDL Cholesterol 50 mg/dl)

Cholesterol 0.5 g/L

Procedure

1. Precipitation

200µl of serum was added to 200µl of Precipitating reagent 2 and was centrifuged at 1500g or 3500-4000 rpm for 10 min.

Clear supernatant was immediately separated and cholesterol content was determined as follows:

2. Cholesterol Assay:

To 1 ml of Reconstituted Reagent , 20µl of the supernatant was added and reading was taken after 5 min of incubation at 37 ^{o}c.

Reference values

Serum HDL-C : 30-70 mg/dl

VLDL AND LDL Cholesterol

These parameters were calculated using Friedwald's formula given below

LDL-c = TC- (HDL-c+VLDL-c)

VLDL-c = TGL/5

RESULTS

The estimated levels of various biochemical parameters namely fasting plasma glucose, serum urea, serum creatinine, serum triglycerides, serum total cholesterol, serum HDL, calculated LDL and serum leptin levels of all the subjects selected for the study irrespective of the grouping are tabulated in Master Table No 2, along with calculated BMI, WHR, exposure to smoking and alcohol all of which are risk factors for essential hypertension. Of the selected subjects, S.No 1- 39 in the Master Table consists of the control subjects and S.No 40-89 consists of elderly males with essential hypertension.

Table No 3 gives the compilation of the levels of biochemical and physical parameters in apparently healthy controls (i.e S.No 1-39 in Table 2). The mean and standard deviation for each parameter is also given in the table.

Table No 4 depicts the compilation of biochemical and physical parameters in the hypertensive subjects (i.e S.No 40-89 in Table 2). The mean and standard deviation of each parameter is also shown in the table. The mean levels of each blood parameter in the two groups are also shown as bar diagrams in Figures 7-15.

To determine how far the levels of biochemical and physical parameters varied between the two groups, the mean levels in controls were compared with that of the hypertensive subjects, which is depicted in Table No 5. The statistical significance was determined using the student's "t" test and Mann-Whitney test was performed for non-normal distribution.

For the discrete variables, such as smoking and alcohol, significance was determined using chi-square test as shown in Table 6 & 7. Association of smoking and alcohol with hypertension was not found.

From the Table 5, it is evident that elderly subjects with high blood pressure had significantly high BMI and leptin levels than healthy controls.

To adjust for the effect of BMI on leptin levels, both the groups were subdivided into three categories – BMI < 19, BMI 19-21, BMI > 21.

Tables 8-10 shows the compilation of biochemical parameters in controls with BMI < 19, 19-21 & >21 respectively. The mean and standard deviation for each parameter is also shown in the tables.

Tables 11-13 shows the compilation of biochemical parameters in hypertensive subjects with BMI < 19, 19-21 & > 21 respectively. The mean and standard deviation for each parameter is also shown.

Table No 14 shows comparison of biochemical parameters between the controls and hypertensives with BMI <19. The statistical significance was determined using t test. Of all the parameters, only serum leptin level was significant with p value < 0.05.

Table No 15 shows comparison of biochemical parameters between the controls and hypertensives with BMI 19-21. The statistical significance was determined using t test. Between the 2 groups, only serum leptin level was significant with p value < 0.05.

Table No 16 shows comparison of biochemical parameters between the controls and hypertensives with BMI >21. The statistical significance was determined using t test. It is evident from the table that leptin levels differed significantly between the 2 groups with p value < 0.05.

Table No 17 shows the results of a univariate logistic regression to analyze the effect of various physical and biochemical parameters as risk factors for the development of hypertension in the elderly. It is evident from the table that hyperleptinaemia and BMI are positively associated with high blood pressure, whereas other parameters such as smoking, alcohol, WHR and lipid profile are not associated with high blood pressure.

Table No 18 shows the results of multivariate regression analysis performed after adjusting for leptin in case of BMI and by adjusting BMI in case of leptin. Only leptin was found to be significantly associated with high blood pressure. The association of BMI and leptin with systolic and diastolic blood pressure is also depicted as bar diagrams in Figures 16 & 17.

DISCUSSION

Analysis of the results obtained in the study starts with scrutinizing the reference range obtained.

The reference ranges of the relevant biochemical and physical parameters of the apparently healthy controls are given below:

Fasting Plasma Glucose	=	81 ± 10.56
Serum Urea	=	23.2 ± 7.73
Serum Creatinine	=	0.98 ± 0.17
Serum Triglycerides	=	111.2 ± 49.61
Total Cholesterol	=	154.4 ± 32.68
Serum HDL	=	38.5 ± 4.53
Serum LDL	=	93.6 ± 33.6
Body Mass Index	=	19.63 ± 2.04
Waist Hip Ratio	=	0.95 ± 0.05

The reference ranges for the various biochemical parameters quoted above for controls are acceptable as they fall within the reference ranges quoted in the methodology adopted for their analysis.

The reference range for serum leptin levels in healthy controls is 3.17 ± 2.84, which is well within the levels given in the kit methodology.

Hence the mean levels of the analysed biochemical parameters obtained from apparently normal geriatric individuals are accepted as valid reference range for the study.

On scrutinising Table 5, where the mean levels of the biochemical parameters in cases are compared with the reference range obtained in the study, it is found that among the analysed parameters, serum leptin levels, BMI and blood pressure are significantly higher in the cases ($p< 0.000$ for leptin and < 0.05 for BMI).

The association of high BMI with elevated blood pressure obtained in the study is consistent with the results obtained in various studies. There are several potential mechanisms linking this association. They are:

* By activation of sympathetic nervous system and rennin-angiotensin-aldosterone system[69,70].

* Increased fatty acid produced in obese patients stimulates aldosterone production independent of renin[71].

* Obesity causes insulin resistance and hyperinsulinemia which leads to hypertension by increasing sodium reabsorption directly, by enhancing sympathetic activity or by increasing responsiveness to angiotensin II in the secretion of aldosterone[72].

Also the association of high leptin levels with hypertension in the cases is consistent with the results from various studies. Several studies suggest the role of leptin in the pathogenesis of hypertension. In animal studies, leptin is found to increase sympathetic nervous activity to the kidneys, hindlimb and adrenal glands[20]. Chronic infusion of leptin in conscious rats was found to increase arterial pressure and heart rate[9]. Leptin deficient ob/ob mice have low

arterial pressure, suggesting a critical role for leptin in maintenance of arterial pressure[73].

Despite some studies showing a natriuretic effect of leptin, leptin appears to elevate blood pressure without increasing natriuresis, thereby adversely shifting the pressure-natriuresis curve[74]. Thus increased renal sympathetic activity combined with decreased natriuresis is likely to produce hypertension[75].

Leptin has also been shown to stimulate endothelial nitric oxide release and cause coronary vasodilatation. However, only sympathectomised rats have a depressor response to leptin, which suggests that leptin-induced sympatho-excitation opposes the direct vasodilatory effect of leptin invivo[76]. Thus given the strong association of leptin and hypertension demonstrated by multiple studies, the invivo vasodilation effects seem to be minimal compared to its sympathetic pressor effects.

In this study, both BMI and leptin levels were found to be associated with hypertension. To check whether the high leptin levels in the cases is independent of the high mean BMI of the cases, it was decided to categorise the controls and cases into three groups based on their BMI, namely "<19", "19 to 21" and ">21".

It was found that in all the three groups leptin was significantly associated with hypertension independent of BMI implying a strong association. Further, a multivariate regression analysis was done adjusting leptin for BMI and BMI in case of leptin. Only Leptin was significantly

associated with hypertension whereas the difference in BMI between the two groups was not significant when adjusted for leptin levels.

The high levels of leptin in the cases might have predisposed these individuals to develop hypertension. Increased activation of sympathetic nervous system combined with decreased natriuresis by high levels of circulating leptin is likely to produce hypertension.

The cause for high leptin levels observed in the cases could be due to

1. Polymorphism in the leptin gene. Shintani et al[11] has demonstrated a highly polymorphic tetranucleotide repeat polymorphism in the 3' flanking region of the leptin gene, thus indicating a genetic association of leptin with hypertension, independent of obesity.

2. Polymorphism in the leptin receptor gene[77].

3. Defect in leptin signal transduction in leptin-expressing neurons in the hypothalamus, thus leading to hyperleptinemia[78]. Since the peripheral actions are preserved, it contributes to the development of hypertension via the mechanisms discussed above.

SUMMARY

Hypertension is one of the common diseases afflicting humans. Because of the associated morbidity, mortality and cost to the society, it is an important public health challenge.

Aging is associated with a metabolic decline characterised by the development of changes in fat distribution, obesity and insulin resistance. All these alterations are associated with a variety of age-related diseases such as hypertension, atherosclerosis and stroke.

It is well recognised that an increased body weight is often associated with metabolic disorders as well as increased blood pressure. Indeed, obesity activates the sympathetic nervous and renin-angiotensin system, all of which have been thought to raise blood pressure.

Recently, a possible role of leptin, a peptide hormone secreted from adipose tissue and involved in the regulation of food intake and satiety, has been implicated in modulating many of these metabolic alterations. Human obesity is characterised by elevated plasma leptin levels and resistance to the metabolic effects of the hormone. The high leptin levels cause increased sympathetic activity in the circulation and at the renal level leading to the development of hypertension.

There is a strong inter-relation between leptin, BMI and other measures of body fat, which has made it difficult to investigate the influence of this peptide on blood pressure. Hence the present study was undertaken to establish the role of leptin in the pathogenesis of hypertension and whether the

association is independent of the level of BMI, body fat distribution and age, as all these factors are, in turn, positively associated with blood pressure.

The study population comprised of 50 elderly hypertensives and 39 elderly healthy controls. The study included physical examination, anthropometric measurements, blood test, medical history, dietary, drinking and smoking habits.

Serum leptin levels were determined using ELISA. Statistical analysis was performed using student's t-Test to detect the association between the selected variables. Multivariate logistic regression was used to determine the role of leptin as a predictor of blood pressure accounting for potential confounders.

It was observed that the serum leptin levels were significantly elevated in the hypertensives compared to the normotensives. The elevated levels of leptin could be due to polymorphism in the leptin gene, its receptor or any alteration in the leptin signaling pathways. The circulating high leptin levels cause increased sympathetic nerve activity and higher plasma levels of adrenaline and nor-adrenaline thus contributing to hypertension.

In this study it was observed that hyperleptinaemia was a significant risk factor for high blood pressure for elderly individuals independent of BMI. This functional data on the direct effect of leptin on blood pressure suggests that the leptin gene and its product, leptin are an attractive target for studies on the mechanism of hypertension and for the development of methods for the prediction, prevention and treatment of hypertension.

CONCLUSION

From the discussion held on the results obtained in the study on leptin, the following conclusion is arrived at regarding the biochemical parameters:

* The serum leptin levels in the control group is 3.17 ± 2.85 which is the reference range for the study.

* Serum leptin levels are significantly elevated in the essential hypertensive cases above the reference range (11.1 ± 7.6) indicating hyperleptinaemia in this group.

* There is no significant change in the levels of lipid parameters between the controls and hypertensive cases in the study.

* The various physical parameters such as BMI and WHR does not seem to significantly correlate with elevated blood pressure.

* There is a significant relationship between plasma leptin levels and blood pressure independent of potential confounders.

SCOPE FOR FURTHER STUDY

* Further studies are needed to determine the precise role of CNS and peripheral effects of leptin in long term blood pressure regulation.

* Studies are needed to investigate whether leptin upregulates the activity of AGT gene and thus contributes to hypertension.

* Studies analyzing the mechanism of leptin receptor signaling by SOCS-3.

* Further studies on polymorphism in the regulatory region of the leptin gene to determine whether the association of leptin with hypertension is primary or secondary.

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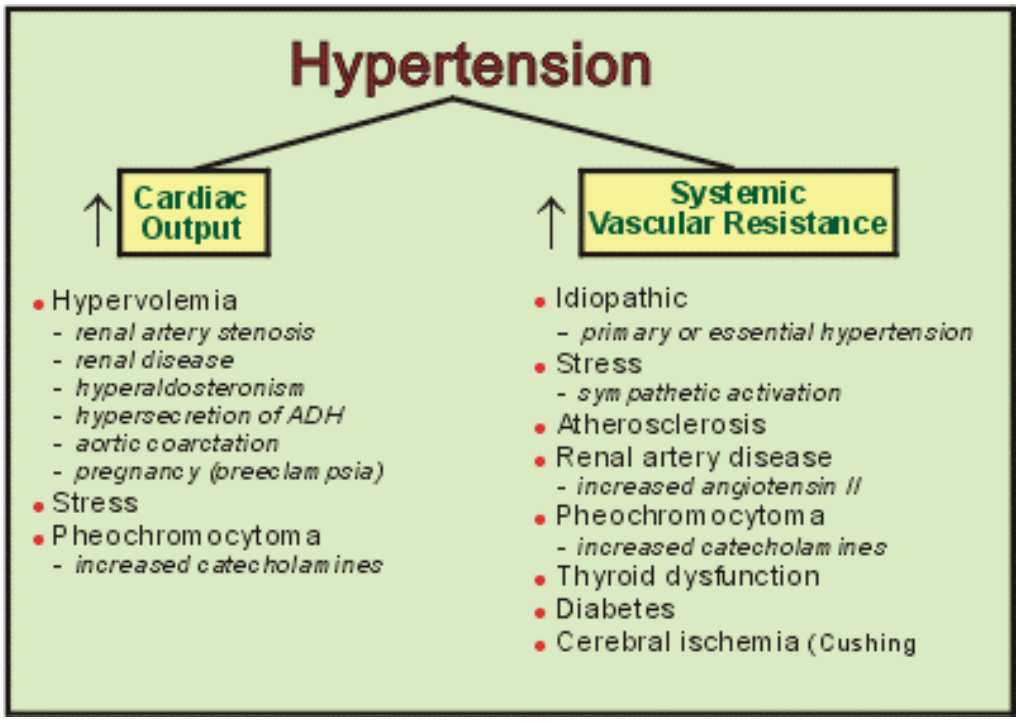
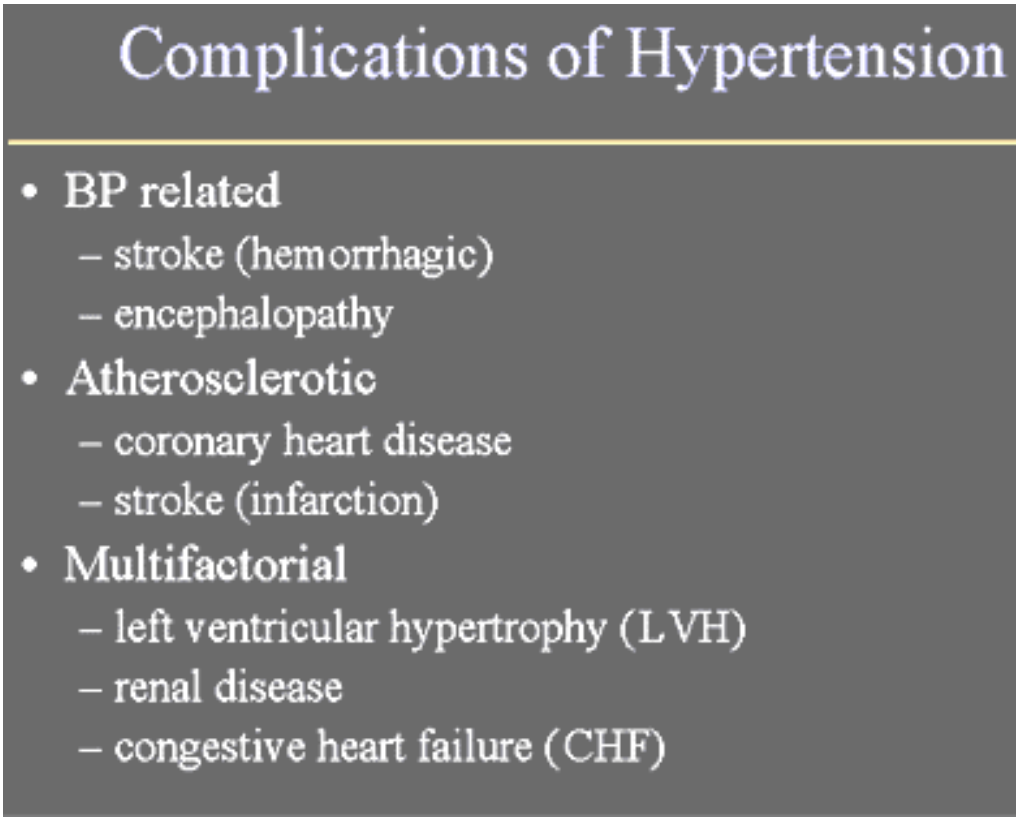
FIGURE - 1



Courtesy : cvphysiology.com

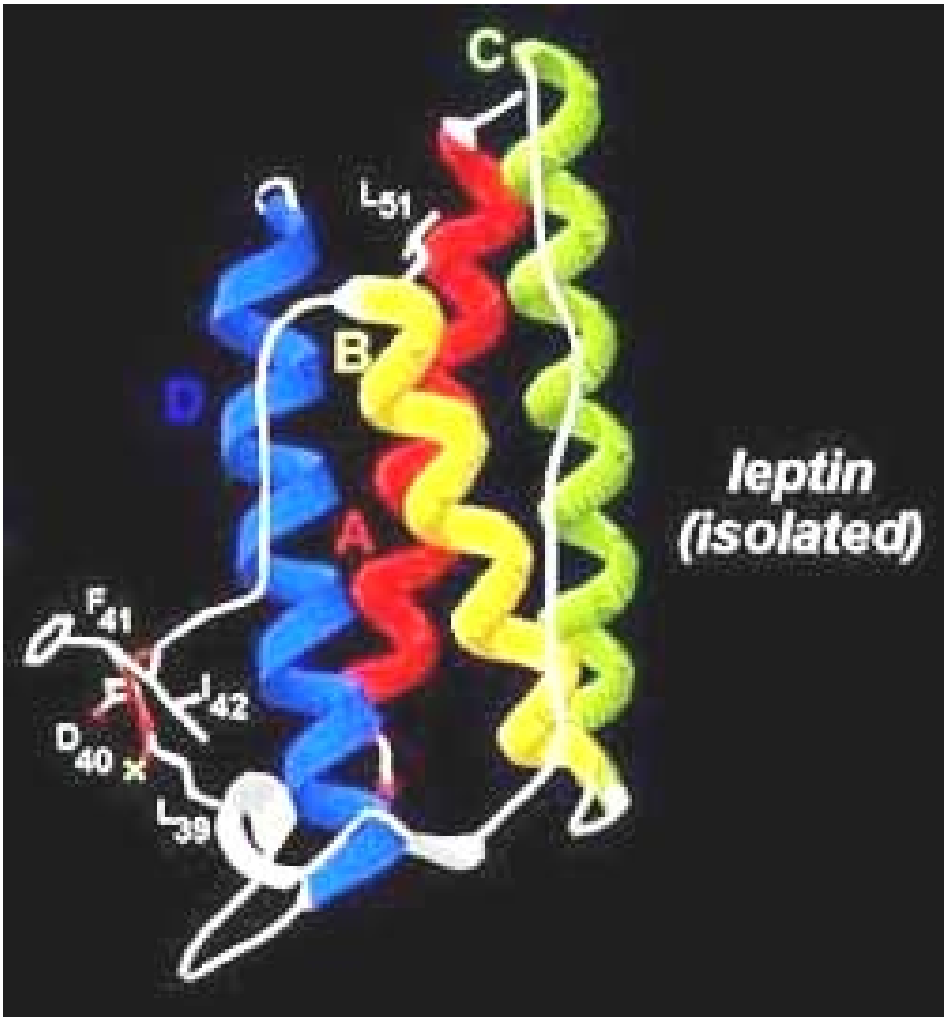
FIGURE - 2



Courtesy : www.medscape.com

FIGURE - 3

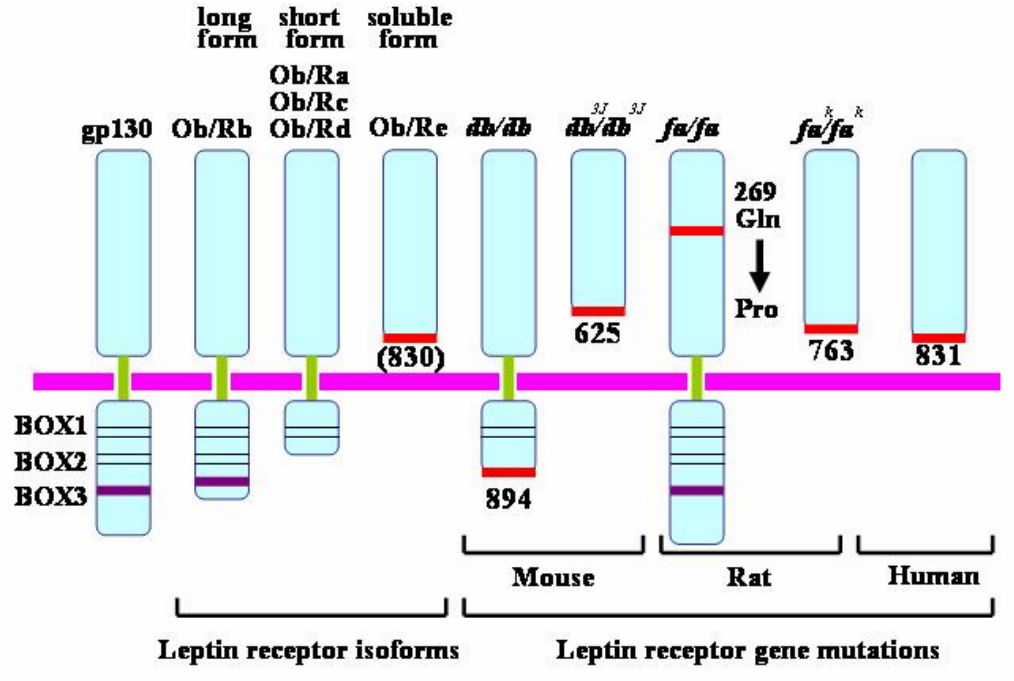
STRUCTURE OF LEPTIN



Courtesy : Wang et al, 2005

FIGURE - 4

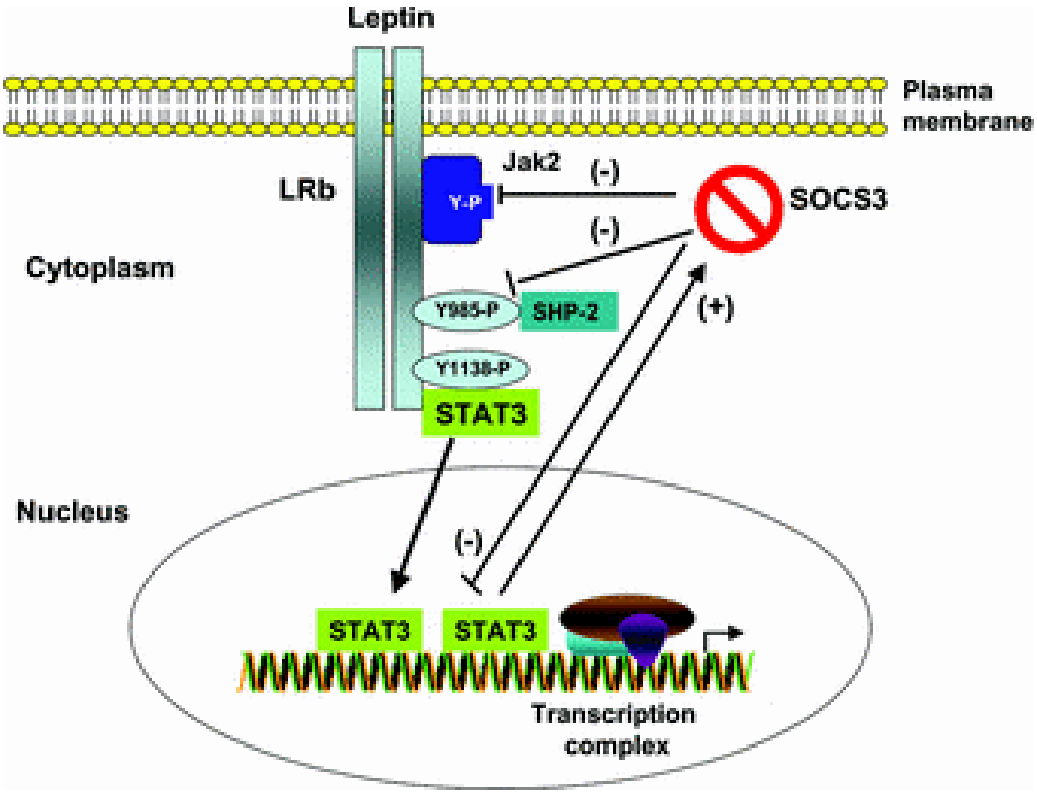
LEPTIN RECEPTOR (OB - R) ISOFORMS AND KNOWN MUTATIONS IN GENETIC OBESITIES



Courtesy : Clioffi et al, 1996

FIGURE - 5

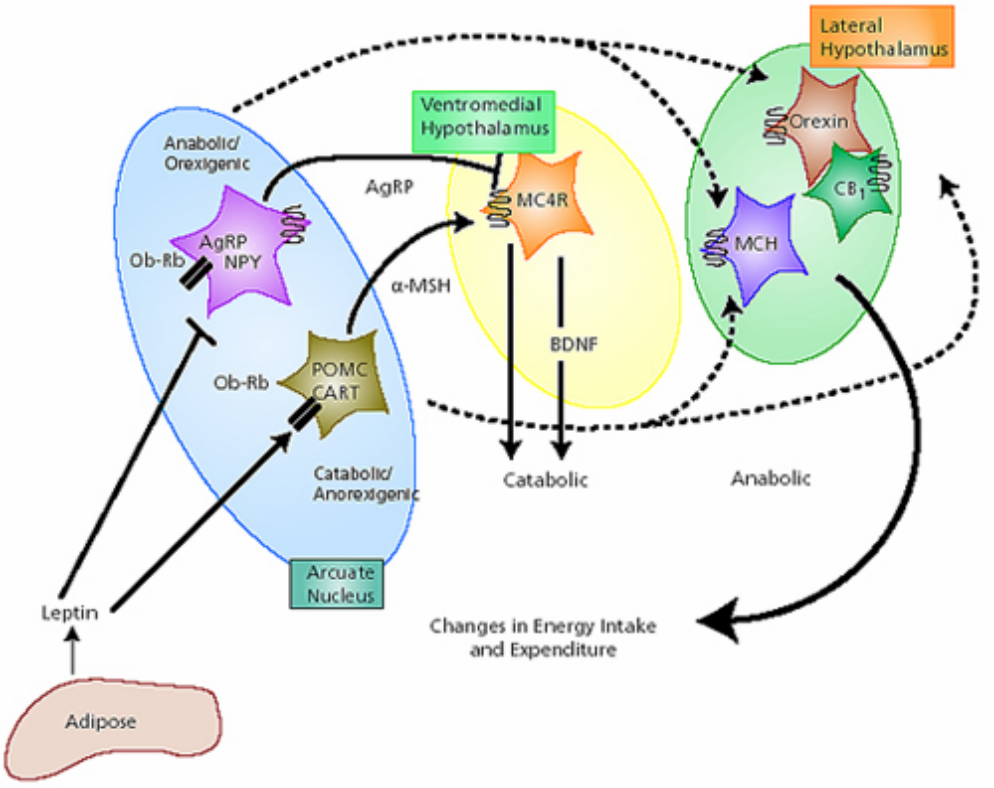
LEPTIN SIGNALING



Courtesy : Malaka et al, 2006

FIGURE - 6

LEPTIN ACTION DOWNSTREAM



Courtesy : www.sigmaaldrich.com

FIGURE - 7

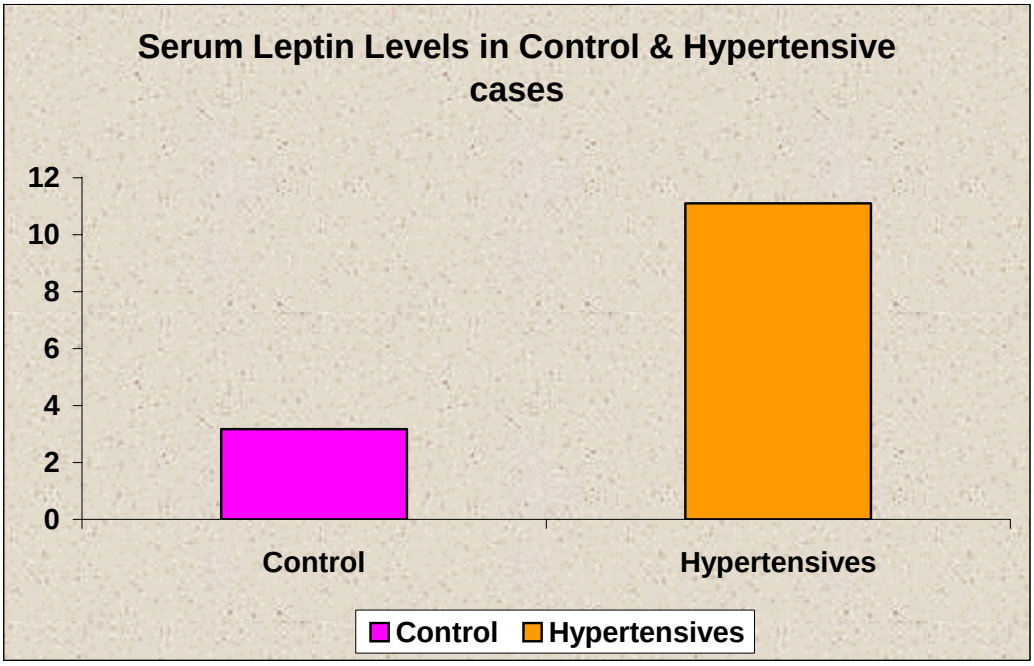
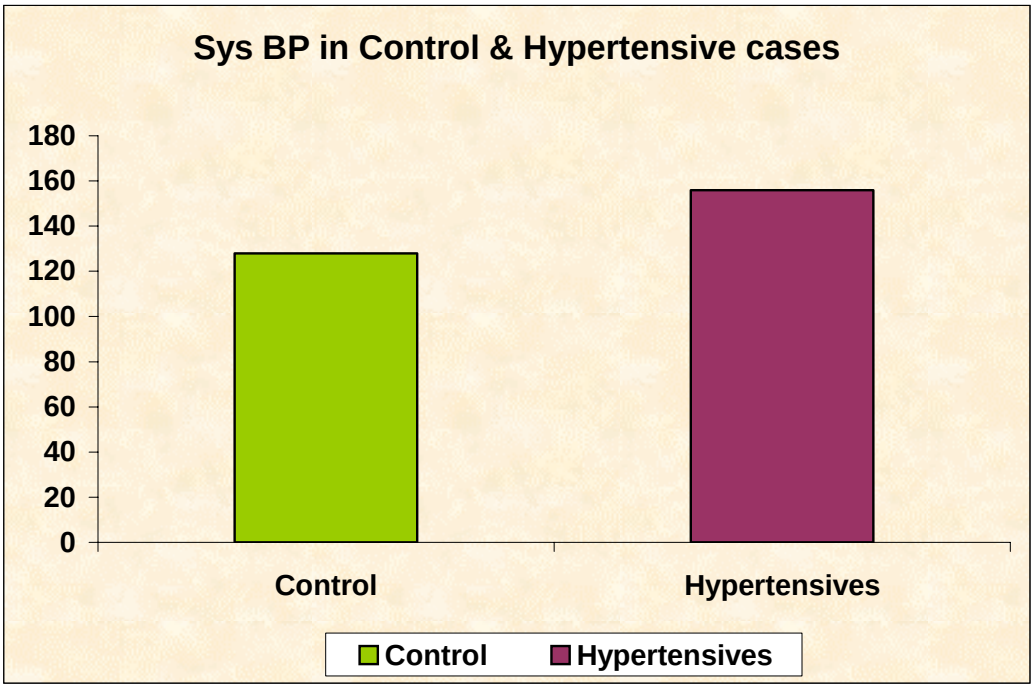


FIGURE - 8



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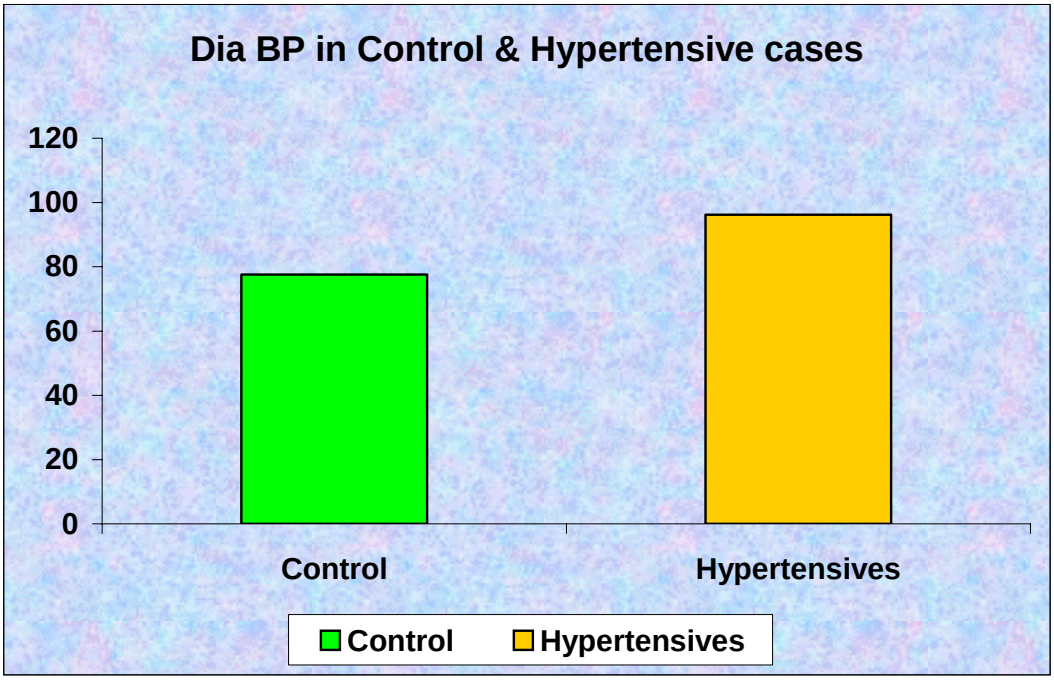


FIGURE - 10

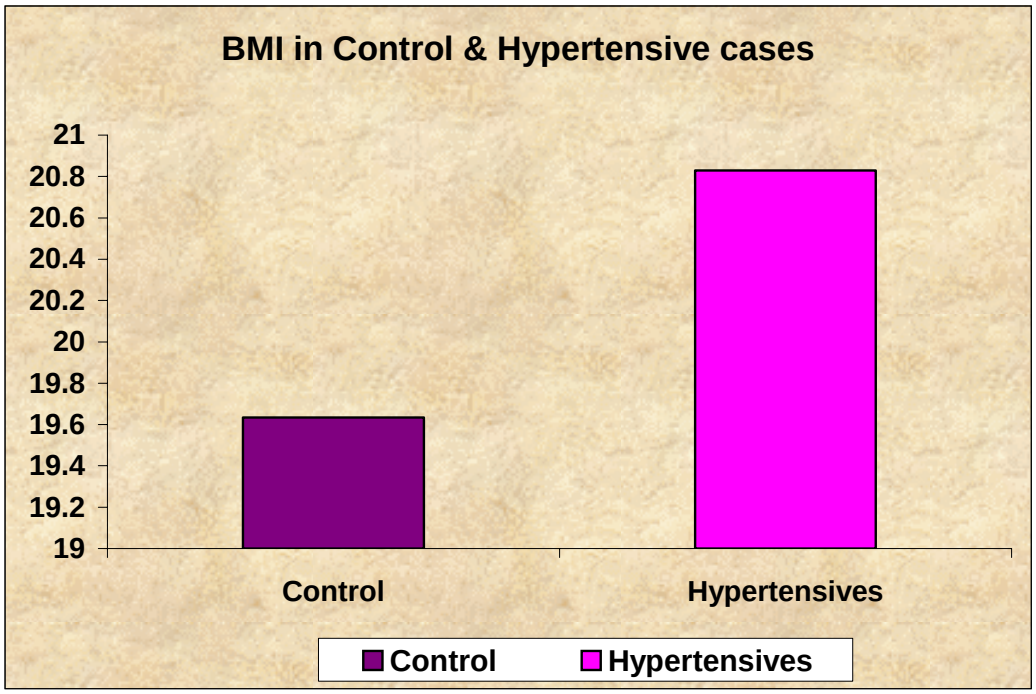


FIGURE - 11

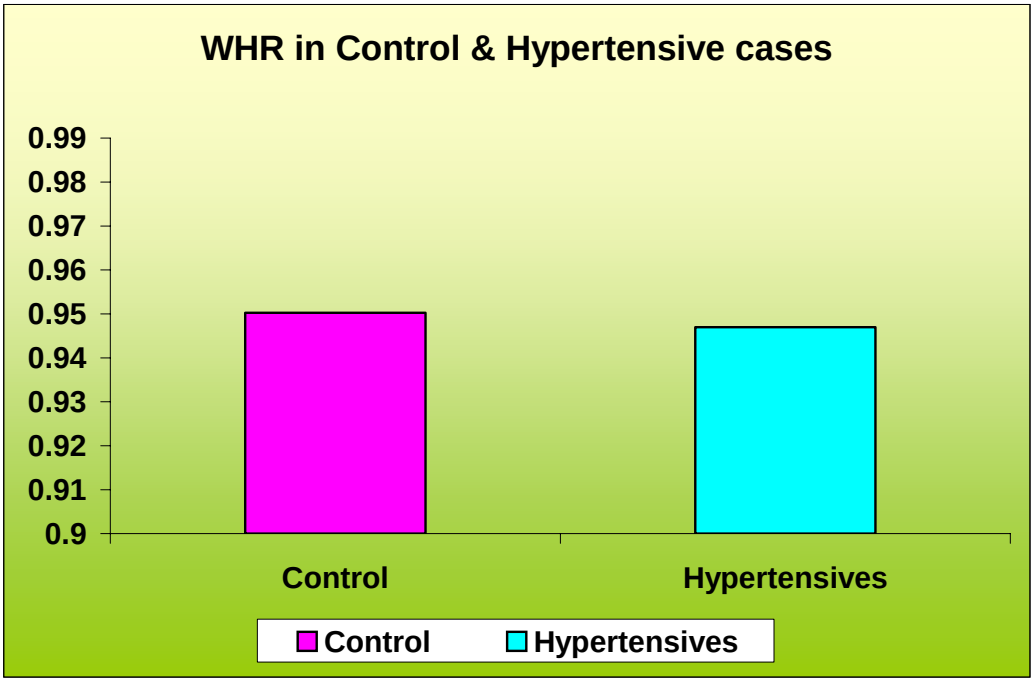


FIGURE - 12

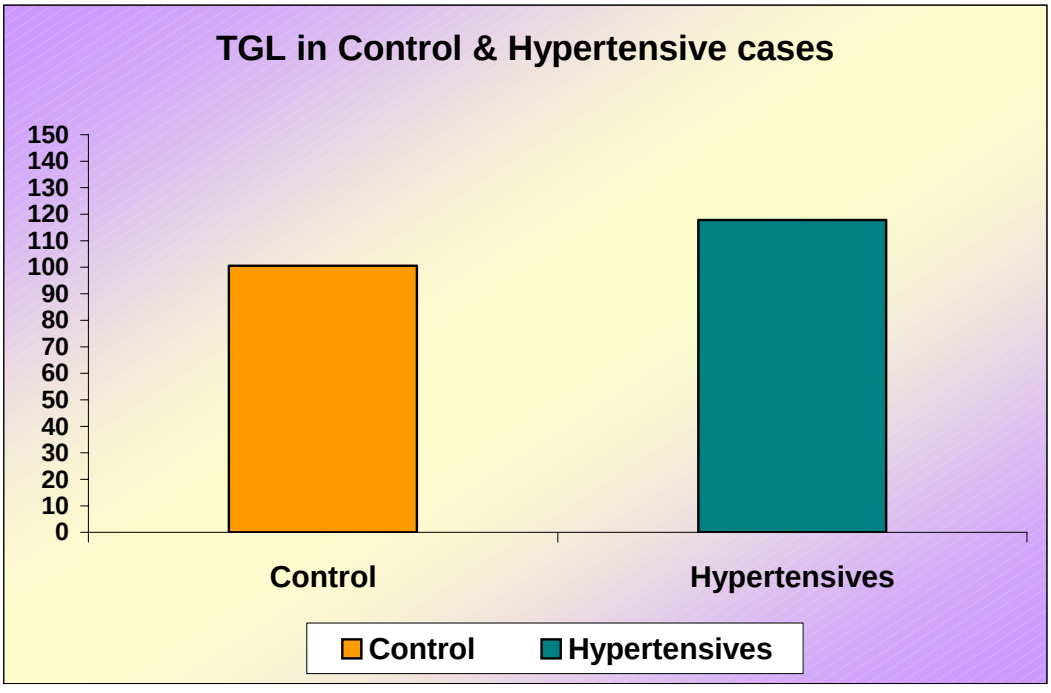


FIGURE - 13

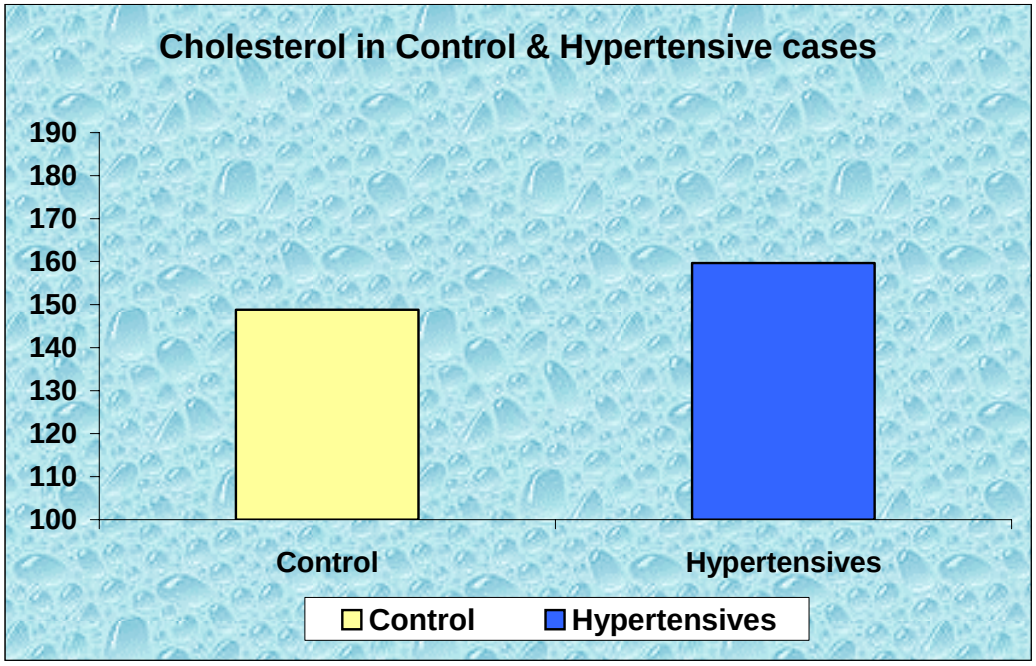


FIGURE - 14

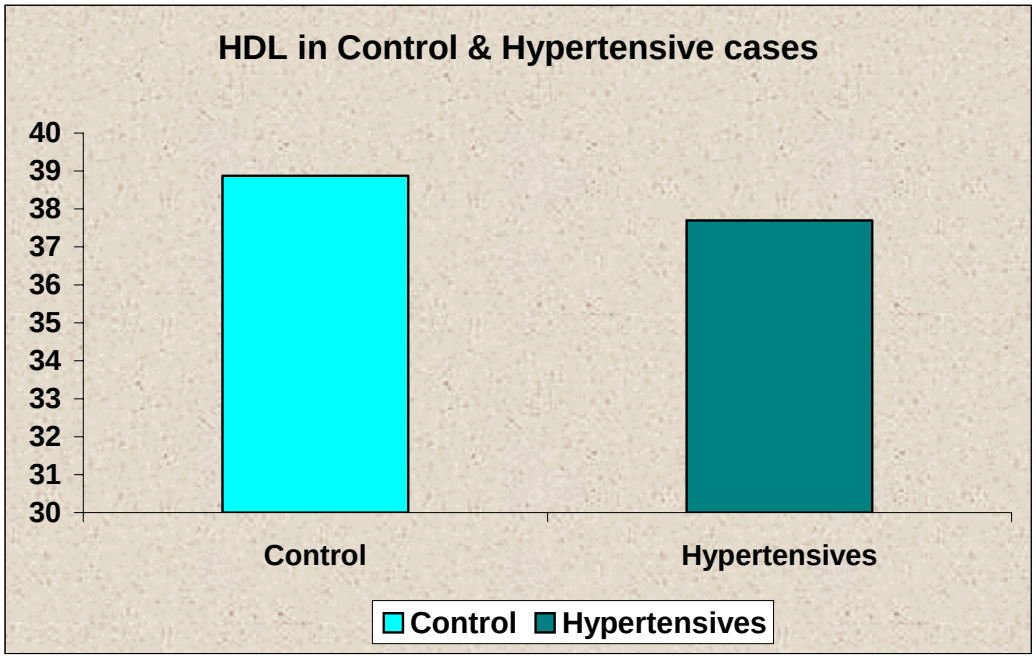


FIGURE - 15

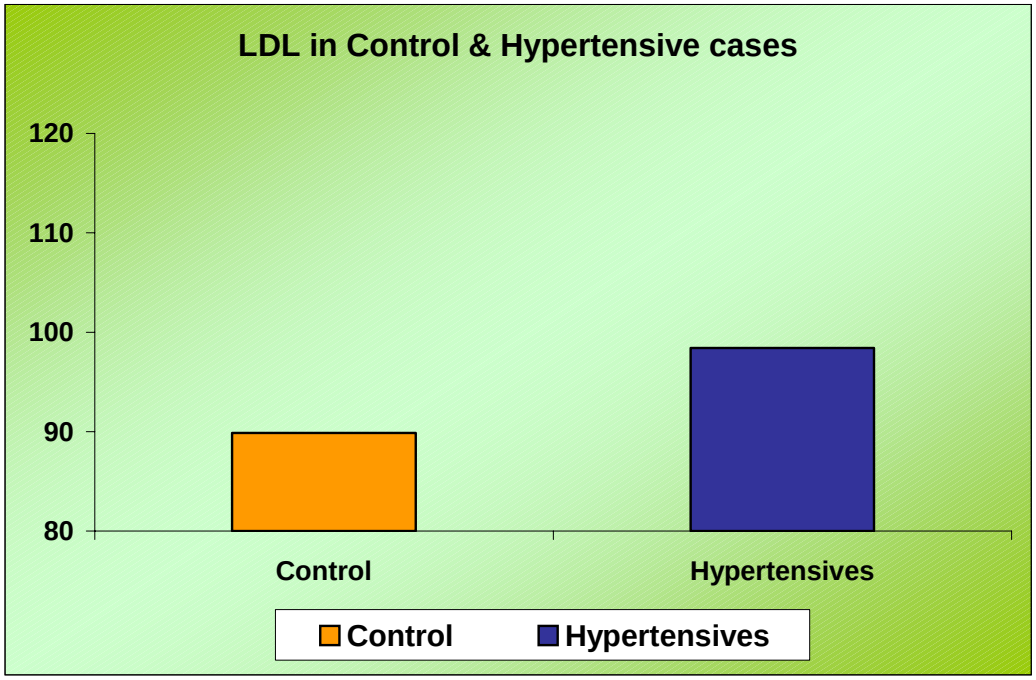


FIGURE - 16

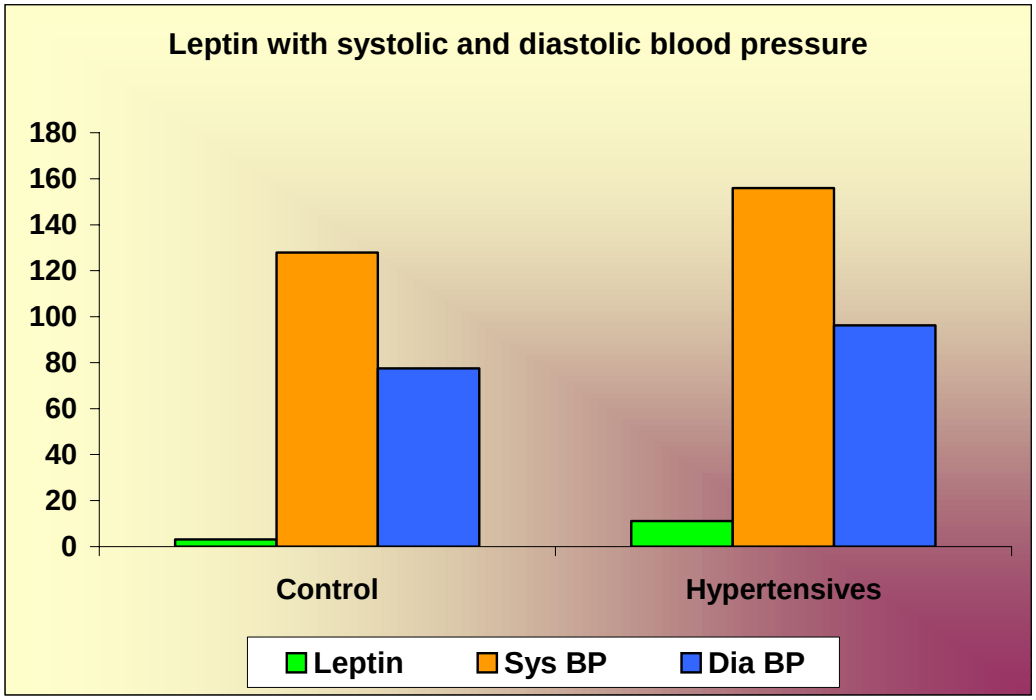
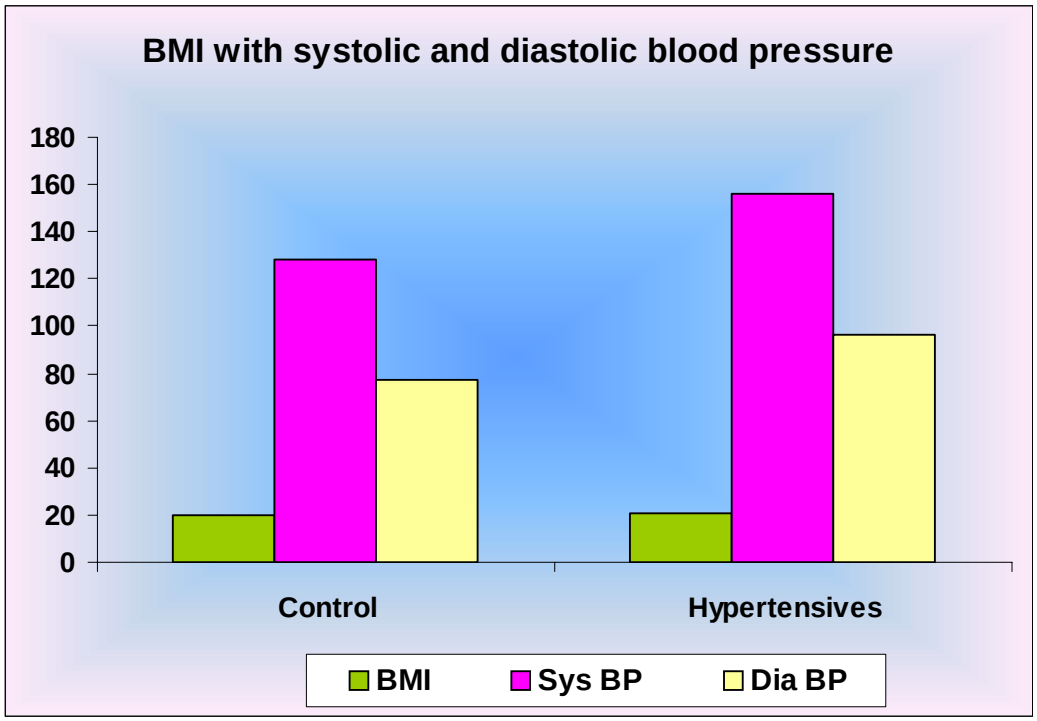


FIGURE - 17


BMI with systolic and diastolic blood pressure
180
160
140
120
100
80
60
40
20
0
Control
Hypertensives
BMI
Sys BP
Dia BP